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THE PULMONARY FUNGUS HAPLOSPORANGIUM PARVUM

STRAIN AND GENERIC RELATIONSHIPS

J. W. Carmichael

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THE UNIVERSITY OF ALBERTA

THE PULMONARY FUNGUS HAPLOSPORANGIUM PARVUM

STRAIN AND GENERIC RELATIONSHIPS

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

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OF MASTER OF SCIENCE

ARTS AND SCIENCE

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by

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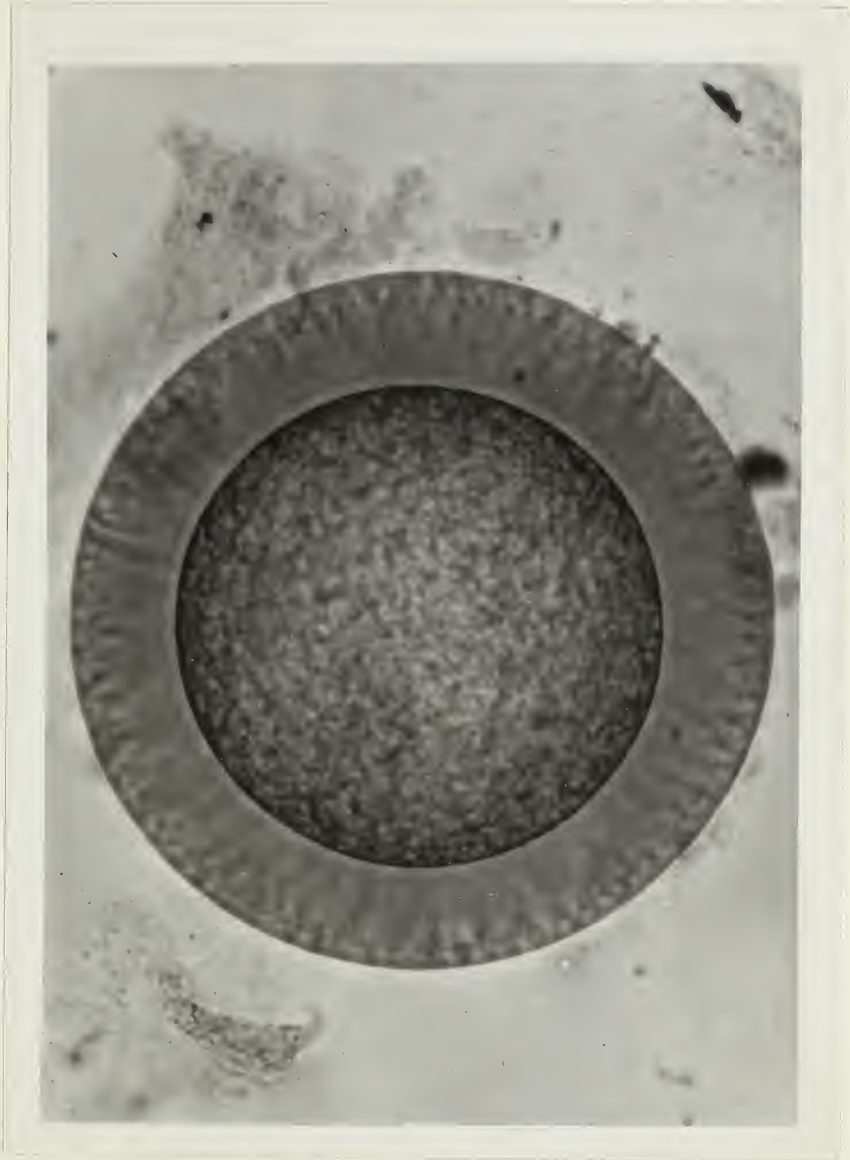

Frontispiece. A single parasitic cell of *Haplosporangium parvum*. x700.

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ABSTRACT

Fifteen strains of Haplosporangium parvum derived from Arizona, Texas, Montana, Idaho, and Alberta animals were studied comparatively. Their colonial morphology reflects to some extent their geographic origin. Northern strains are slower growing at room temperature than southern strains, their spores are slightly larger, they form chlamydospores at a lower temperature. Unlike most southern strains, they form nodules when injected subcutaneously into guinea pigs and mice. One Montana strain was like northern strains in some particulars and like southern strains in others. All strains are similar in microscopic morphology. The differences among strains do not justify splitting H. parvum into two or more species.

The possession of erect fertile hyphae and chains of conidia will necessitate the exclusion of H. parvum from the genus Haplosporangium Thaxter. H. parvum is probably not related to Coccidioides, or any other Phycomycete. Its closest affinity is with Blastomyces dermatitidis.

THE PULMONARY FUNGUS HAPLOSPORANGIUM PARVUM STRAIN AND GENERIC RELATIONSHIPS

In 1942 Emmons and Ashburn (12), in an attempt to find animal vectors of human fungus diseases, examined wild rodents in the vicinity of San Carlos, Arizona. They found that the lungs of 25 of their 300 animals harboured sporangia of Coccidioides immitis, the causative agent of coccidioidomycosis (valley fever, desert rheumatism, San Joaquin fever) in man. In addition to this fungus, the lungs of 100 of the animals contained spherical bodies about 14 microns in diameter. When cultured on nutrient medium these bodies gave rise to colonies of a previously undescribed fungus. Microscopic examination of these colonies showed delicate, frequently branching hyphae and subspherical, hyaline conidia.

In some of the animals, from which this fungus only was obtained in culture, endospores were observed in the bodies in the lungs. For this reason Emmons considered the new fungus to be closely related to Coccidioides immitis, and perhaps even a mutant of that species. Unlike Coccidioides however, it formed conidia in culture. He therefore placed it in the genus Haplosporangium Thaxter of the Phycomycetes, and described

it as a new species, Haplosporangium parvum.

Ashburn and Emmons (2) infected guinea pigs, monkeys, and mice with pure cultures of H. parvum. The bodies produced in the lungs of laboratory mice were somewhat larger (35-40 microns) than those seen in wild mice. They did not find endospores in 200 experimental animals.

In Alberta in 1946, Dowding (8) discovered in the lungs of mice and a squirrel, large, thick-walled, spherical bodies attaining a diameter of 300 microns. To her surprise, these bodies when cultured produced a mold indistinguishable from the Haplosporangium described by Emmons and Ashburn.

When cultures of Alberta strains of H. parvum were maintained at 37°C, their conidia enlarged to form spherules which she considered to be homologous with those in the lungs of animals. No endospores were seen in the lungs of Alberta animals nor in the bodies formed in culture. She called the bodies chlamydospores.

Coccidioides immitis produces arthrospores in culture which are readily blown about by air currents. Blastomyces, Histoplasma and Haplosporangium parvum produce adhesive conidia in culture which are not readily detached. For this reason, Dowding considered that H. parvum was more closely related to Histoplasma and Blastomyces than to Coccidioides.

Since then, Jellison has found the large chlamy-

dospores of Haplosporangium in many Montana animals, including rock rabbits, weasels, and skunks as well as beaver and various other rodents. No endospores were observed in these animals. His recent paper (15) describes these discoveries and reviews reports of Haplosporangium from Minnesota, Texas, California, and British Columbia.

There is a tremendous difference between the size of the chlamydospores found in the lungs of Arizona and Texas rodents and those found in the lungs of Montana and Alberta animals. Chlamydospores of southern strains are 10-14 microns in diameter, while chlamydospores of northern strains are 110-390 microns. Because of this difference in size it has been suggested by Emmons (personal communication) and Jellison (15) that the Haplosporangium occuring in Montana and Alberta is a different species than the one originally described from Arizona.

In order to determine more clearly the relationship between northern and southern strains, fifteen cultures of H. parvum were obtained from Arizona, Texas, Montana, Idaho, and Alberta animals, and their characters compared on culture media and in laboratory animals. A study of these strains has persuaded the author that they should be retained as one species. Further, it has disclosed features which suggest that this mold was wrongly placed in the Phycomycetes.

MATERIALS AND METHODS

Strains 1 to 3 came from animals with small chlamydospores in the lungs, while strains 4 to 15 came from animals with large chlamydospores (with the possible exception of Montana strain 14 which was not seen in the host (15) and was atypical in experimental animals). The seven Alberta strains came from prairie, parkland, and forest regions of the Province. Three of the four Montana strains were collected not from rodents, but from carnivores. Strains 1-3 were kindly sent by Dr. C. W. Emmons, strains 11-15 by Dr. W. L. Jellison. Details of the sources of the strains are shown in Table I.

Cultures were grown on; Sabouraud's agar (Difco), Littman's agar (Difco) (17, 18), corn meal agar (4), and blood agar. Room temperature varied from less than 10°C at night, to more than 30°C on hot days. Cultures at elevated temperatures were made by transferring fragments from 2-8 week old Sabouraud-agar-slant cultures to blood agar plates. These were incubated in the inverted position, and water was added to the lids as necessary to prevent drying.

Microscopic examination was made on material mounted in 85% lactic acid 2 parts, and Lugol's iodine 1 part.

The spores often varied considerably in size in a single mount, especially chlamydospores. Therefore,

TABLE I

FIFTEEN STRAINS OF HAPLOSPORANGIUM PARVUM

STRAIN	LOCATION	HOST	COLLECTOR AND NUMBER
1	Arizona	?	Emmons ?
2	Arizona	?	Emmons 2370
3	Texas	pack rat	Emmons 2372
4	Alberta	deer mouse	Dowding 184
5	Alberta	deer mouse	Dowding 19
6	Alberta	deer mouse	Dowding 10
7	Alberta	deer mouse	Dowding 17
8	Alberta	deer mouse	Dowding 190
9	Alberta	deer mouse	Dowding $F_2 9$
10	Alberta	deer mouse	Dowding 50-59
11	Montana	weasel	Jellison 27258
12	Montana	muskrat	Jellison 27242
13	Montana	skunk	Jellison 27128
14	Montana	weasel	Jellison 27274
15	Idaho	deer mouse	Jellison 27097A

to avoid personal influence in selection, all spores in focus at one time were measured. The spores were considered to be isodiametric and were measured without reference to their point of attachment to the conidiophore.

Nuclei were stained by the hydrolysis-basic fuchsin method of DeLamater (7).

Animal injections were made with spore suspensions which were prepared by adding about 10 c.c. of sterile, normal saline to Sabouraud-agar-slant cultures (2-8 weeks old) and scraping the surface growth with a loop. The bottles were then shaken gently and the suspensions checked microscopically to be sure spores were present. Animals were injected subcutaneously on each side of the abdomen using 0.2 c.c. of suspension for each injection.

Since the Haplosporangium spores remained localized after injection, and developed in the subcutaneous tissues, their development could be followed without resorting to embedding and sectioning. Small pieces of subcutaneous tissue from the injection sites were teased apart and examined in 10% potassium hydroxide or lacto-phenol cotton-blue, or for microcultures, on Sabouraud's agar. Tissue sections were also cut and stained in the usual manner. The writer is indebted to Dr. D. O. W. Waugh of the Department of Pathology for examining the slides.

PART 1

STRAIN RELATIONSHIPS

CULTURES COMPARED AT ROOM TEMPERATURE

Colony Types

On the basis of colonial morphology on Sabouraud's agar, cultures of the fifteen strains of Haplosporangium can be classified into four types (Figure 1), as follows--

Granular type colonies have a short nap of aerial mycelium (one millimeter deep or less) which has a plasterlike appearance reminiscent of Trichophyton mentagrophytes. The surface of the colony is heaped and thrown into folds. Spore production in these colonies is moderately high. Strains 1, 4, 5, 6, 8, 9, 10, and 15 have produced granular colonies.

Cottony type colonies have a dense, usually white felt of aerial mycelium. The texture and appearance of these colonies is very close to Histoplasma capsulatum. Spore production is low or absent. This colony type has been seen in Arizona strains 1, 2, and 3, and Alberta strain 7.

Floccose type colonies have a loose, buff-colored aerial mycelium covering the surface of the colony. The aerial hyphae adhere in tufts, giving the appearance of sheep's wool. These colonies have high spore production and there are many secondary spores. Floccose colonies were seen mainly in Montana and Idaho strains, but also in Alberta strains 6 and 8.

Glabrous type colonies often have a central tuft of spines, but no other aerial mycelium. They are moist

Figure 1. Four colony types. Left to right; granular, cottony, floccose (with glabrous central zone), and glabrous (with some aerial mycelium).

and circular, flat or smoothly raised towards the centre. The firm hyphal mat is almost the color of the agar.

This type of growth is usual in all young cultures, but often persists, especially when the agar is moist and the temperature is high. Cultures of all strains have been observed to remain in this condition for at least two months when transferred in hot weather. Old cultures of other types sometimes revert to the glabrous condition by collapse of the aerial mycelium. Many cultures maintain glabrous zones or sectors.

These four colony types are intergrading. Also one strain may show more than one type of colony when transferred under different conditions. Strain 1, for example, has produced glabrous, granular and cottony types of colonies. In general however, granular colonies are typical of Alberta strains, cottony colonies of Arizona strains, and floccose colonies of Montana strains, while glabrous colonies are produced by all strains under some conditions.

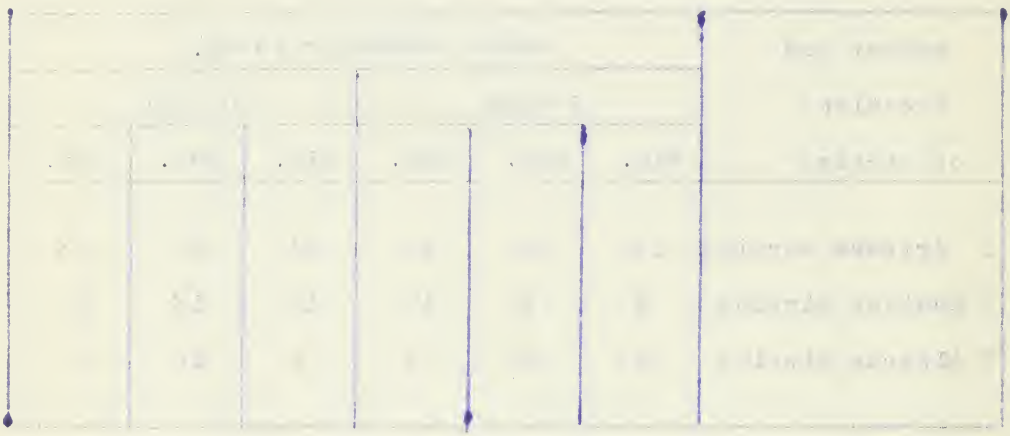
Growth Rate

The growth rate of Haplosporangium was obtained by measuring the diameter of young colonies after five and after ten days incubation (Table II). The three southern strains grew more rapidly than the Alberta and Montana strains; in fact after both five and ten days, the smallest southern colonies were larger than

TABLE II

GROWTH RATE ON SABOURAUD'S AGAR

number and location of strains	colony diameter in mm.					
	5 days			10 days		
	min.	ave.	max.	min.	ave.	max.
3 Arizona strains	12	12	12	24	26	28
5 Montana strains	3	9	11	16	18	22
7 Alberta strains	3	6	9	9	16	20



any produced by northern strains. Colonies of Alberta strains were smaller, on an average, then those of Montana strains, but individual colonies showed exceptions.

Number and Size of Spores

When the fifteen strains of Haplosporangium parvum were maintained in the laboratory on Sabouraud-agar-slants and transferred every three months, most of them consistantly produced large numbers of spores. Four strains (the three southern strains and Alberta strain 7) became sterile. Transferring to fresh medium usually had no effect. Sometimes however, a single transfer in a series of sterile ones would inexplicably produce many spores. Two southern sterile strains (#1 and 2) were transferred to sterile mouse and rabbit dung in a moist chamber. Under these conditions both strains sporulated abundantly.

Eight of the fifteen strains were selected for a study of their spore size. Table III shows that when the average spore sizes are compared by geographic groups, Alberta strains average very slightly larger than Montana strains, and both are somewhat larger than Arizona strains. When the average spore sizes of the individual strains are examined however, it can be seen that it would be impossible to "place" with assurance an unknown strain with a spore diameter of, say, 3.3 microns or 3.5 microns.

TABLE III

AVERAGE SPORE DIAMETERS OF

EIGHT HAPLOSPORANGIUM STRAINS

Geographic group	Strain number	Average diameter of 20 spores in microns	Diameters by geographic groups		
			Minimum	Average	Maximum
Alberta	8	4.2			
"	10	3.6	2.5	3.8	5.5
"	4	3.6			
Montana	14	4.1			
"	13	3.5	2.5	3.6	5.0
"	12	3.2			
Arizona	1	3.2			
"	2	3.1	2.5	3.2	4.0

From three-month-old Sabouraud-agar-slant cultures at room temperature.

Colony Color on Littman's Agar

When the fifteen strains of H. parvum were transferred to Littman's agar (containing dextrose, peptone, crystal violet, and streptomycin (17, 18)), it was found that certain strains produced distinctly grey colonies in contrast to the usual white or buff colored ones. The three southern strains (#1, 2, and 3), two of the Alberta strains (#5 and 8), and two of the Montana strains (#14 and 15) showed this grey coloration of the aerial mycelium. The grey color did not seem to depend on geographical location and its significance is not understood.

CULTURES COMPARED AT HIGHER TEMPERATURE

When the conidia of Alberta strains of H. parvum are maintained at 37°C they enlarge to form chlamydospores similar to those found in the lungs of infected animals (8). To determine if other North American strains behaved similarly, fragments from cultures of four Montana strains, three southern strains, and an Alberta control strain were incubated at 37°C. The diameter of 20 spores of each strain was measured at intervals.

Table IV shows that three of the Montana strains and the Alberta strain formed chlamydospores, while conidia of the fourth Montana strain (#14) did not enlarge appreciably.

TABLE IV

SPORE ENLARGEMENT AT 37°C

Source	Strain	Average spore diameter in microns			
		at rm. temp.	2 days @ 37°C	5 days @ 37°C	10 days @ 37°C
Alberta	10	3.6	4.1	7.6	18.1
Montana	13	3.5	3.2	7.0	26.0
Montana	11	-	3.9	8.3	8.6
Montana	12	3.2	3.7	7.4	15.2
Montana	14	4.1	4.6	4.5	4.7

The three southern strains (#1, 2, and 3) did not form chlamydospores. Their conidia swelled slightly and germinated within two days to give rise to the mycelial phase (Figure 13F). Colonies formed at this temperature were atypical, being compact, moist, and tan-colored. The hyphae became closely septate and conidia were not formed.

Since southern strains seemed more resistant to heat, they were incubated at a higher temperature, 40°C. At this temperature southern strains too formed chlamydospores. Table V shows the extent of enlargement at 40°C of two southern strains as compared with two Alberta and two Montana strains. All strains except Montana 14 enlarged to about the same extent.

Representative strains from each geographic area (#2, 5, 13, and 15) were incubated at 45°C. No enlargement or growth was observed after seven days. The conidia remained viable however and germinated when returned to room temperature. One strain tested (#8) was killed in 24 hours at 50°C.

The following generalizations from these experiments seem justified. At 37°C Alberta and Montana strains form chlamydospores whose size varies greatly with the strain. Arizona strains, which grow in the mycelial phase at 37°C, form chlamydospores at a slightly higher temperature. Northern strains of H. parvum can be distinguished from southern strains by their lower

TABLE V

SPORE ENLARGEMENT AT 40°C

Source	Strain	Average spore diameter in microns			
		at rm. temp.	2 days @ 40°C	6 days @ 40°C	12 days @ 40°C
Alberta	10	3.6	5.2	8.5	9.7
Alberta	8	4.2	4.5	5.1	11.9
Montana	13	3.5	4.8	6.0	9.0
Montana	14	4.1	4.3	6.2	6.6
Arizona	2	3.2	5.6	9.3	10.3
Arizona	3	-	-	11.3	12.1

optimum temperature for chlamydospore formation.

EXPERIMENTAL INFECTIONS COMPARED

To compare their development in animals, two Arizona strains, three Montana strains and five Alberta strains of H. parvum were injected subcutaneously into guinea pigs and white mice.

Northern Strains

All of the animals inoculated with Alberta and Montana strains (23 guinea pigs and 6 mice) developed subcutaneous nodules. These appeared within 2-10 days after inoculation and sometimes persisted as long as seven months (the longest time any animal was observed). They were apparent as firm swellings at the sites of injection (Figure 2). Excised nodules ranged up to two centimeters in diameter.

The nodules were situated in the dermis or the underlying muscle. Typically they consisted of an abscess, or a small granuloma, surrounded by an area of subacute and chronic inflammatory reaction and fibrosis. Foreign-body giant cells were usually numerous. Occasionally epithelioid cells were present and the changes resembled a tuberculous type of response; but usually (apart from the presence of the organism) there was nothing to suggest a specific type of reaction. Figure 3 shows a portion of the wall of one of the abscess cavities.

Figure 2. Nodules on the abdomen of a guinea pig. Two months after subcutaneous injection of spores of Haplosporangium parvum.

Figure 3. Section through a nodule showing tissue reaction and chlamydospores. x75.

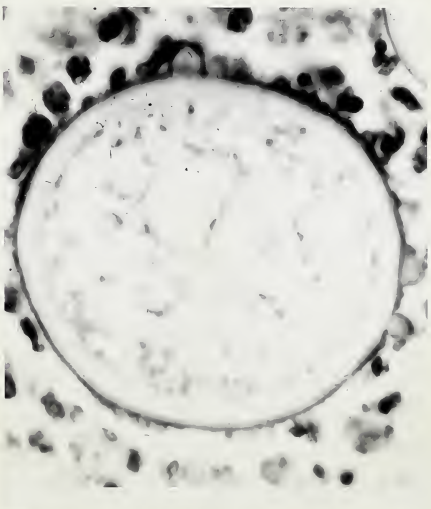
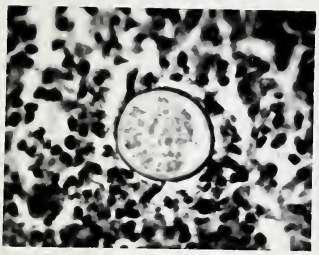


Figure 4. A disintegrating chlamydospore with "blisters" formed by the separation of the outer and inner walls. x750.

The central part of the nodule usually contained chlamydospores of the fungus. These cells reached their maximum size in 2-6 weeks. In some of the older nodules (two months or more) the fungus cells were disintegrating or had been completely absorbed. Figure 4 shows a chlamydospore beginning to disintegrate; the outer wall is separating form the inner wall, forming blisters on the surface of the cell. Sometimes, when mounted in potassium hydroxide, the outer wall separates completely from the inner, as in Figure 13B. This figure recalls Jellison's illustration of H. parvum from the lungs of a rock rabbit (14), and suggests that chlamydospores of H. parvum disintegrate in wild animals in the same way as in experimental animals. A later stage of disintegration, in which the outer wall has completely disappeared, is shown in Figure 8.

Chlamydospores of Montana strain 14 were only 6-9 microns in diameter after six days in the animal and closely resembled those seen in the lungs of Arizona rodents. Figure 9 shows these small spores in giant cells. For comparison, spores of Alberta strain 8 are shown after the same length of time, and at the same magnification in figure 10.

Spores of other northern strains were similar to those seen in the lungs of naturally infected Alberta and Montana animals. They were generally smaller however. In one animal the maximum diameter was only



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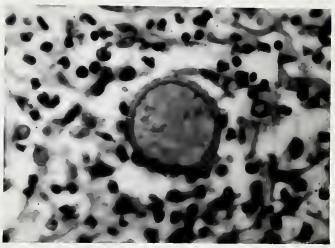

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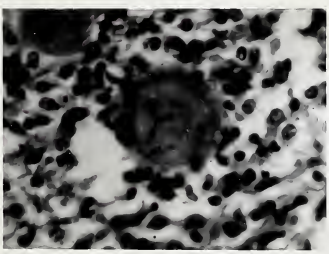

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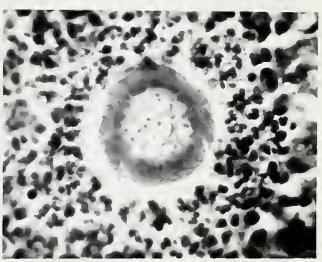

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Figures 5-8. Four stages in the disintegration of chlamydospores. 5, normal cell. 6, blisters forming. 7, outer wall mostly disolved (surface view). 8, outer wall disolved, inner wall greatly thickened and irregular. All x335.

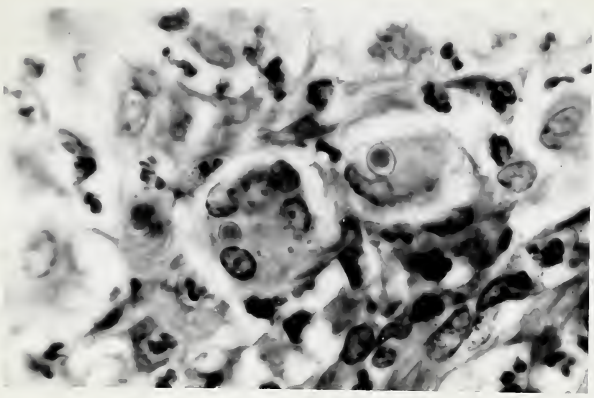


Figure 9. Chlamydospores of Montana strain 14 after 6 days in guinea pig. Tissue section. x750.

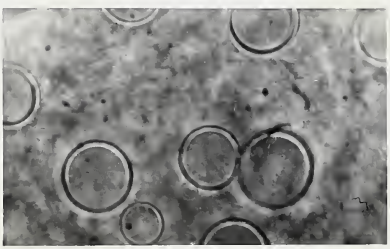


Figure 10. Chlamydospores of Alberta strain 8 after 6 days in guinea pig. Compare size with Figure 9. Lacto-phenol cotton-blue preparation. x750.

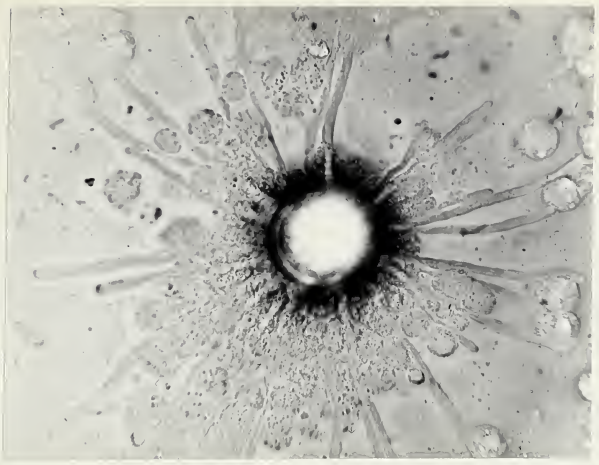
55 microns after two and one-half months. The largest cells seen in any experimental animal were 175 microns.

In one series of four guinea pigs injected with the same spore suspension (Alberta strain 8), all developed typical nodules on both sides of the abdomen within ten days. In three of the pigs the nodules disappeared after only two months. Microscopic examination at this time showed that most of the chlamydospores had disintegrated and were absorbed. In the fourth animal, both nodules remained at their maximum size (about 10 mm. in diameter) for seven months. When the animal was killed at this time the nodule contained many large chlamydospores which proved viable when cultured.

Southern Strains

Of 11 animals (8 guinea pigs and 3 mice) inoculated with southern strains, only two pigs developed nodules; moreover these disappeared in less than a month. In comparison with northern strains, spores of Arizona and Texas strains were rapidly absorbed by the animal. Usually, spores had disappeared from the site of injection in as short a time as ten days.

Generally speaking, the condition of chlamydospores in animals appeared to depend upon three factors; the time elapsed since injection, the strain used, and the resistance of the individual animal.



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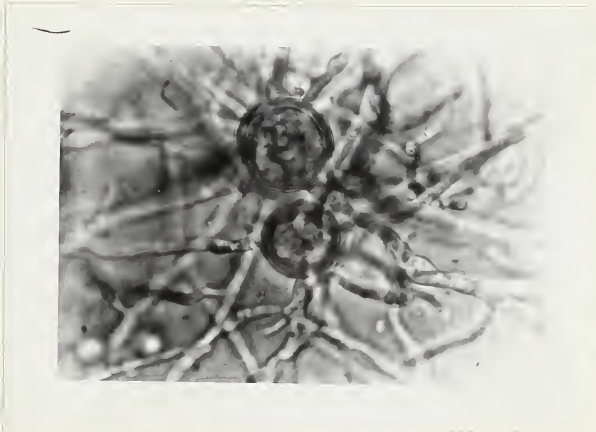


b

Figure 11. Germinating chlamydospores from nodules teased out in water. a, living cell in water. b, stained with cotton blue. Note septa in the hyphae. x335 and 750.

No evidence of internal disease was found in any of the animals inoculated subcutaneously. Neither was Haplosporangium obtained from the lungs, liver or spleen when these organs were cultured from five animals. Cultures from nodules however produced an abundant growth of H. parvum in pure culture. Chlamydospores from nodules germinate by the extrusion of several germ tubes (Figure 11).

It now seems established that H. parvum does not multiply in the host, or cause a progressive disease (2, 8, 15). The presence of chlamydospores in the lung results usually in a slight, non-specific type of reaction. Further, the fungus cells in the lung are probably destroyed in time. We may consider H. parvum a feebly pathogenic and probably accidental parasite in the lungs of small mammals. The parasitic cells are extremely large and easily seen. If it were not for this, H. parvum would be listed as a contaminant along with certain species of Penicillium and Beauveria which can also be regularly isolated from rodent lungs (unpublished data from surveys).

DISCUSSION

When the strains were compared with respect to six differential characteristics they separated readily into three groups.

Arizona and Texas strains: colony type, cottony; relative growth rate, 26 (mm. in 10 days); relative spore size, 3.2 microns; chlamydospores formed in culture at 40°C, in animals 10-40 microns; not usually forming nodules when injected subcutaneously.

Montana and Idaho strains: colony type, floccose; relative growth rate, 18; relative spore size, 3.6 microns; chlamydospores formed in culture at 37 and 40°C, in animals to 400 microns; forming nodules when injected subcutaneously.

Alberta strains: colony type, granular; relative growth rate, 16; relative spore size, 3.8 microns; chlamydospores formed in culture at 37 and 40°C, in animals to 300 microns; forming nodules when injected subcutaneously.

The microscopic morphology (which is described in Part 2) is similar in all three groups. Other than the size differences noted above, they cannot be separated on this basis.

One strain, Montana 14, did not fit the three-group classification. It did not form typical chlamydospores in culture at either 37 or 40°C, its conidia were too large for the Montana-Idaho group, its chlamydospores in experimental animals were small, and its growth rate was faster than that of the other Montana strains. This strain is intermediate between the Arizona and Montana groups in some respects, and intermediate between the Montana and Alberta groups in other respects.

It is evident that H. parvum could be split into either two species (northern strains and southern strains), or three species (Arizona and Texas strains, Montana and Idaho strains, and Alberta strains). However, from what is known of the distribution of this fungus, it is likely that other intermediate forms may be found in the States to the north and east of Arizona which would invalidate these species. The scattered reports from Minnesota, British Columbia, and California indicate that H. parvum is very widespread in its distribution.

All strains studied form a complex which seems homogeneous enough to be covered by one specific name. The known distribution of H. parvum rather suggests that the three groups are ecologic units within the species which have become adapted to different climates. That the main distinguishing features are differences in degree of response to temperature, supports this idea. Alberta strains and Montana strains are sufficiently different in the sum of their characters to be recognized as forms. The Alberta-Montana strain complex and the Arizona-Texas strains are more sharply demarcated and could be given varietal distinction. The writer suggests however that the addition of names to the literature should be delayed until the distribution and taxonomic position of this fungus are better known.

PART 2

GENERIC RELATIONSHIPS

MORPHOLOGY

Although a morphological study of H. parvum revealed no structural differences among the fifteen strains, it did reveal characters which have a bearing on the taxonomy of the species as a whole.

When H. parvum is grown in petri dishes on corn meal agar the fertile hyphae are at first erect or ascending (Figure 12). They arise from the subsurface mycelium or from trailing hyphal ropes. They are either simple, or branched at right angles to give a tree-like habit (Figure 13C). These fertile hyphae are not highly specialized and do not differ markedly from the vegetative mycelium; in fact there is a gradation from completely sterile hyphae to those which bear many spores.

The spores themselves are borne on short conidiophores which are usually very narrow (Figure 13H). However some of the conidiophores are swollen distally as in figure 13I. After the conidium is partially mature, one or more secondary conidiophores often grow out from it. These in turn produce secondary conidia (Figure 13A). This process may be continued, and occasionally chains of three or rarely four conidia are formed. In some cultures secondary conidia are very numerous (Figure 14)

The conidia are liberated by fracture or dissolution of the conidiophore wall at a point between the

Figure 12. Section through a corn-meal-agar plate culture of H. parvum showing spores on erect fertile hyphae. Photographed in air. x75.

Figure 13. (See next page.) Hyphae and spores of H. parvum. A and C, various strains showing secondary conidia (hyphae with septa not shown are in air). B, part of a chlamydospore (in potassium hydroxide) that has developed in a guinea pig for 5 months. a, outer wall, which has separated completely from the inner wall b. c, protoplast containing globules which stain with Sudan IV. D, young fertile hypha. E, spores germinating at room temperature (Montana strain). F, spores germinating at 37°C (Arizona strain). G, old fertile hyphae with spores detached, showing remnants of conidiophores. H, hyphae with slender conidiophores. I, with ampuliform conidiophores. J, conidium showing remnant of conidiophore. K, showing point of attachment to a secondary spore. L, hyphal nuclei. a and b, young hyphae. c, older hypha. All figures, including B, to same scale.

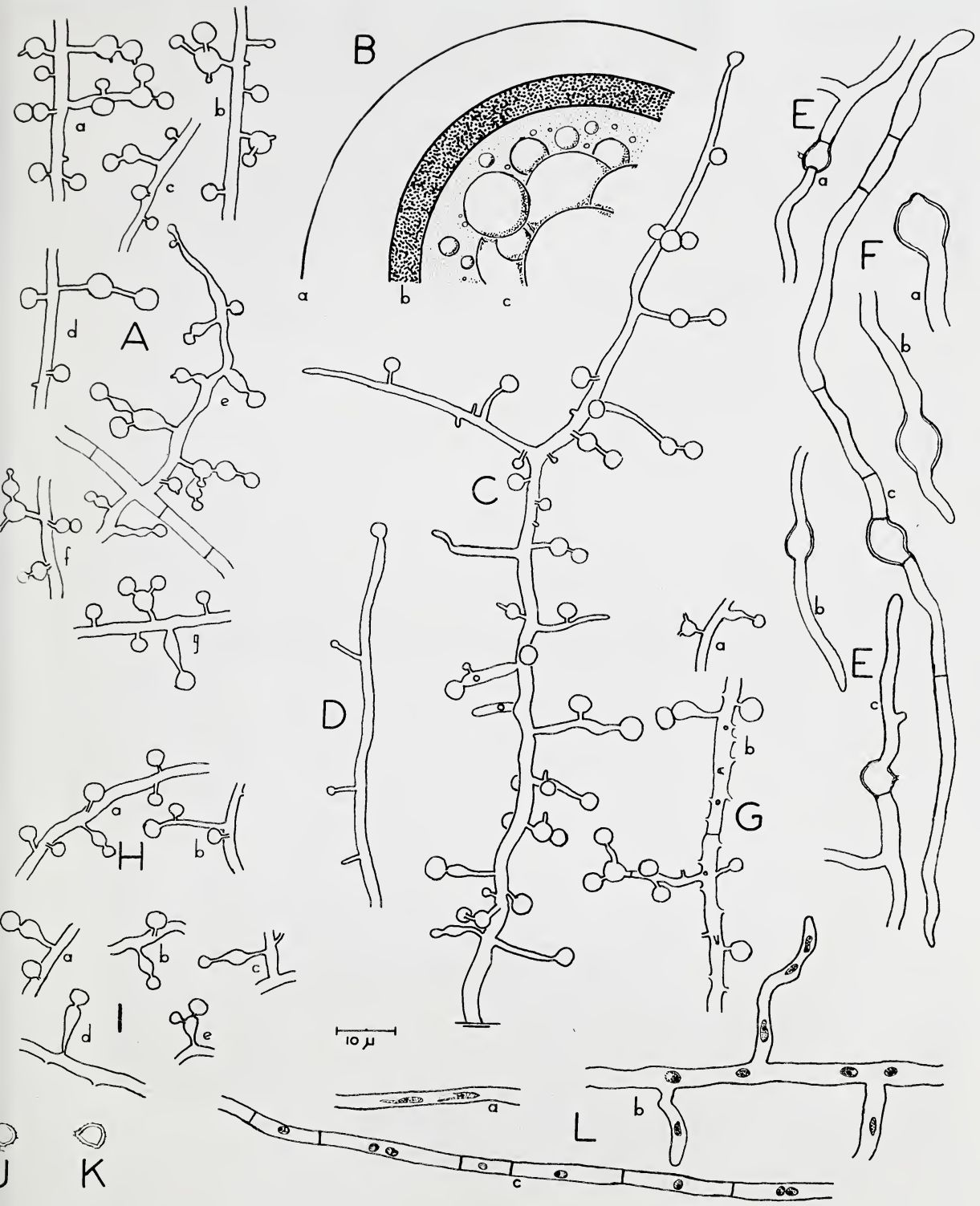


Figure 13. See previous page for description.

Figure 14. Secondary spores of H. parvum. Photographed in air in microculture. x250.

basal septum of the conidium and the parent hypha. This leaves scars along the fertile hyphae (Figure 13G). No septum is visible in spent conidiophores and the open end is apparently plugged with a refractile substance (Figure 15B,d). The ragged end of the broken conidiophore can often be seen at the base of the freed conidium (Figure 13J and 15B,c). Conidia which have produced secondary spores can be seen to have two or more points of abscission (Figure 13K).

Conidia of H. parvum have a double wall. There is a thin, refractile outer layer which is often spiny, and a thicker, less refractile inner layer (Figure 15B,c). The tremendous enlargement of the spores in animals, and their subsequent disintegration makes the two layers easily visible (Figure 13B and 4).

Conidia placed on Sabouraud's agar at room temperature begin to germinate in 14 hours by the formation of a single germ tube. Later a second germ tube grows from the opposite side of the spore (Figure 13E).

The nuclei of H. parvum are fairly evenly spaced along the hyphae and are never seen two abreast (Figure 13L,a and b). In older hyphae and in the conidia there are one or two nuclei per cell (Figure 16 and 13L,c). In both northern and southern strains, chlamydospores formed in culture become multinucleate as they enlarge (Figure 17). The large chlamydospores of Alberta and Montana strains in animals contained thousands of nuclei (Figure 18 and 19).

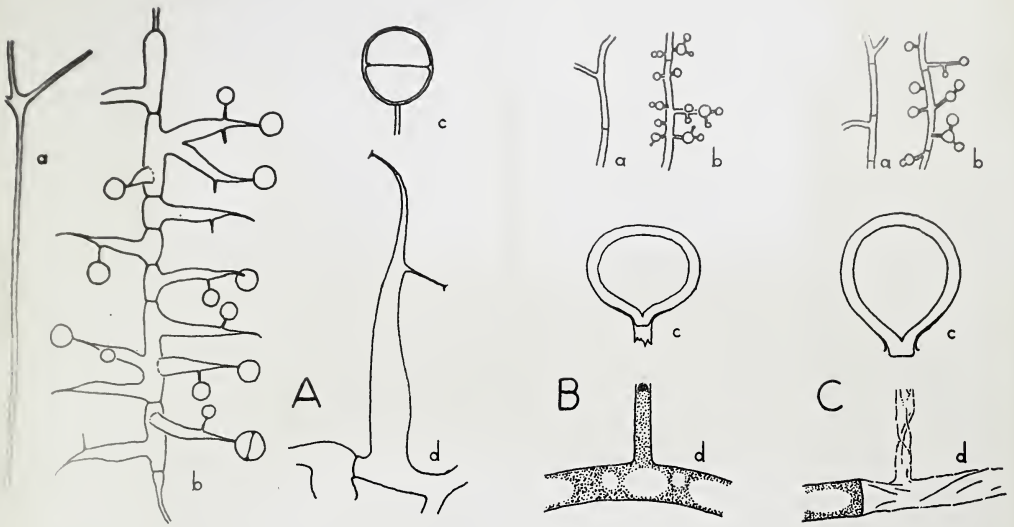


Figure 15. Three fungi compared. A, H. bisporale, after Thaxter (21); B, H. parvum; C, B. dermatitidis. a, vegetative hyphae; b, fertile hyphae; c, spores; d, sporophores after release of spores, showing collarettes (in A), protoplasmic plug (in B), and dissolving walls (in C). Drawings a and b are at the same magnification for each of the three species.

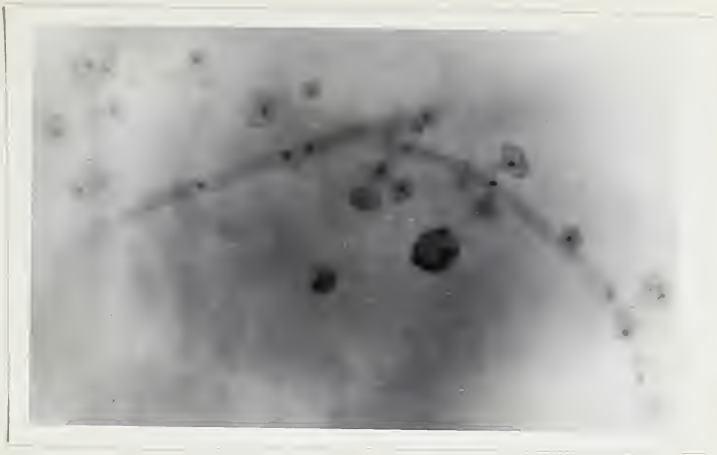
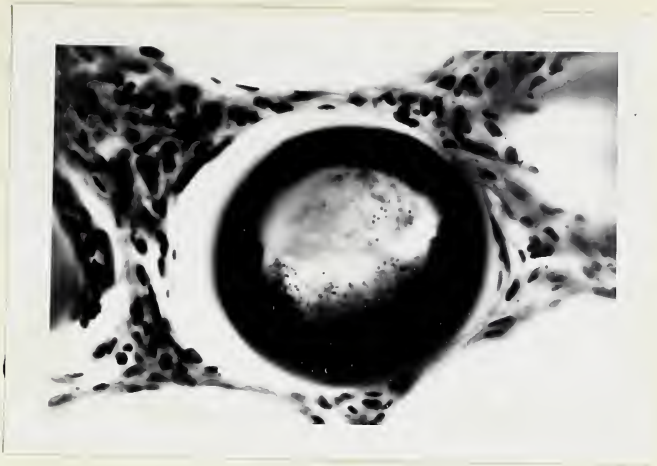


Figure 16. Nuclei in hyphae and conidia of H. parvum. x1500.

Figure 17. Nuclei in chlamydospores of an Arizona strain in culture at 40°C. x1500.



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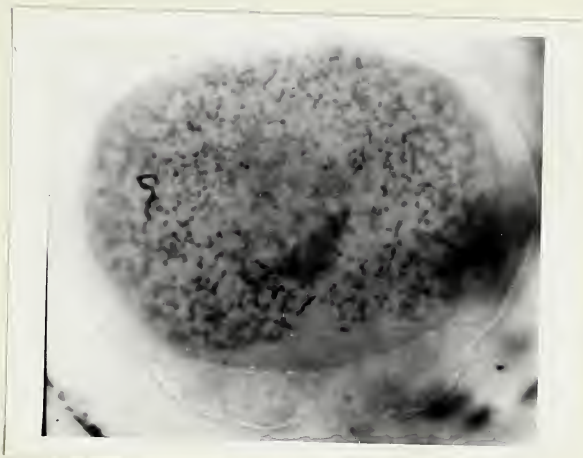

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Figures 18 and 19. Nuclei in chlamydospores from nodules. 18, x335; 19, x750.

DISCUSSION

Relationship with Haplosporangium Thaxter

Thaxter (21) described the genus Haplosporangium as similar to Mortierella Coemans, but differing in the possession of prostrate, highly specialized fertile hyphae (Figure 15A,b). H. parvum does not possess such specialized hyphae. It differs also from Thaxter's two species in having stipitate, often catenate conidia. Furthermore, Thaxter's drawings (Figure 15A,d) show that in his species (as in Mortierella) there is a collarette left at the tip of the sporangiophore after release of the spores. In H. parvum the spores are released by fracture or dissolution of the sporophore itself; consequently no collarette can be seen, and the spores often have remnants of the conidiophore attached to the stipitate portion. In H. parvum the fertile hyphae are erect and the conidiophores do not taper. In the other three species of Haplosporangium (H. bisporale Thaxter, H. decipiens Thaxter, and H. lignicola Martin (19)) the fertile hyphae are prostrate and the sporangiophores taper.

Therefore, H. parvum does not belong in the genus Haplosporangium as constituted by Thaxter, and should be removed as soon as a more suitable placement can be made.

Relationship with Coccidioides

Another possible point of attachment with the Phycomycetes is through Coccidioides immitis Rixford and Gilchrist. Earlier (12), it was considered that H. parvum was probably related to Coccidioides for the following reasons. 1, The two fungi were found associated in the same hosts and locations. 2, In his first series of wild rodents, Emmons observed endospores in the lungs of animals from which only H. parvum was obtained in culture. 3, Parasitic cells of the two fungi are indistinguishable at some stages of their development. 4, Skin-test antigens of the two fungi may produce cross reactions.

Recently discovered facts, on the other hand, argue against a close relationship between H. parvum and C. immitis. 1, C. immitis is probably restricted to certain semi-arid areas (19), while H. parvum, as we now know, is very cosmopolitan in its range and hosts (15). 2, Ashburn and Emmons (2) were unable to find endospores in 200 animals infected with pure cultures of Haplosporangium. In Montana and Alberta, where Coccidioides does not occur, endospores have not been found in wild animals, nor has endosporulation been observed in experimental animals inoculated with these strains. There is still no evidence to dispute Dowaing's statements (8) that, "Chlamydospores of Haplosporangium parvum do not bud. They do not proliferate in any way.

They merely enlarge." (In the host). 3, The mold fungi pathogenic for man and animals present a reduced morphology in the host, many occuring a single cells when parasitic (e.g. Histoplasma, Blastomyces, Sporotrichum, Phialophora, Hormodendrum, and Candida). That H. parvum and C. immitis should both occur as single spherical cells under these conditions is not surprising, and does not necessarily imply relationship. 4, Although infection with H. parvum produces a sensitivity detectable with a coccidioidin skin-test (11), Howell (13) has shown that such non-specific reactions are probably due to high antigen concentrations. Bearing in mind the differences in the life-cycles, morphology, and distribution of the two fungi, we are brought to believe that any similarities are coincidental with their parasitism and do not denote relationship.

Relationship with the Phycomycetes

It has been suggested that the conidia of H. parvum are monosporous sporangioles (12). Their double spore wall is not a safe criterion of this, since many higher fungi show this feature. Sclerotinia vaccinii (16), Thielaviopsis bassicola (16), many rusts (1, figure 25 a, f, h, and o; 6, figures 6-10), Scopulariopsis brevicaulis, and Blastomyces dermatitidis (writer's observations) have double-walled spores. The smut, Mykosyrinx cissi, has spores with two cells which are enclosed by a common outer wall, which at maturity ruptures, freeing the spores (3).

a

b

Figure 20. Blastomyces dermatitidis; secondary conidia. b, showing a chain of four spores. x150.

If H. parvum is removed from the genus Haplosporangium and is not related to C. immitis, then it has no obvious connection with the Phycomycetes. In the absence of a sexual stage it would then be placed in the family Moniliaceae of the Fungi Imperfecti.

Relationship with Blastomyces and Histoplasma

Fertile hyphae of H. parvum, Blastomyces dermatitidis Gilchrist and Stokes, and Histoplasma capsulatum Darling are almost indistinguishable. The demonstration of secondary conidia in H. parvum, B. dermatitidis (Figure 20), and H. capsulatum (10) provides another point of similarity among fungi that already have been considered a natural group (8,9), and sets them apart from most other imperfect forms. Of the three, H. capsulatum is distinct with its tuberculate macroconidia, while B. dermatitidis and H. parvum are more closely akin (Figure 15B and C).

Whether or not to place H. parvum and B. dermatitidis in the same genus depends on the taxonomic weight given their parasitic phases (It should be remembered here that B. dermatitidis and B. brasiliensis (Splendore) Conant and Howell are wrongly placed in the genus Blastomyces Cost. and Roll. (5)). An acceptable classification of Haplosporangium parvum and other dimorphic fungi must await a better understanding of relationships in the Moniliaceae as a whole.

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